Cytological characterization of potato – *Solanum etuberosum* somatic hybrids and their backcross progenies by genomic in situ hybridization

F. Dong, R.G. Novy, J.P. Helgeson, and J. Jiang

Abstract: Four somatic hybrids derived from a diploid wild species *Solanum etuberosum* and a diploid tuber-bearing *Solanum* clone 463-4, together with five BC_1 and three BC_2 plants, were analyzed by genomic in situ hybridization (GISH). None of the four somatic hybrids had the expected chromosome constitutions, i.e., 24 chromosomes from each fusion parent. Either one chromosome from *S. etuberosum* or one from the potato parent 463-4 was lost in the hybrids. Three BC_1 plants had exactly one set of *S. etuberosum* chromosomes. The other two BC_1 plants either had one extra or one fewer *S. etuberosum* chromosome, possibly because their somatic hybrid parents had an extra or had lost one *S. etuberosum* chromosome. The presence of one set, or close to one set, of *S. etuberosum* chromosomes in all BC_1 plants suggests a preferential pairing and segregation of the *S. etuberosum* chromosomes in the somatic hybrids. Two of the three BC_2 plants had 52 chromosomes, deviating significantly from the expected chromosome number of 48. These results suggest poor pairing between *S. etuberosum* and *S. tuberosum* chromosomes in the BC_1 plants. The present study demonstrates the importance of combining GISH and DNA marker analysis for a thorough characterization of potato germplasm containing chromosomes from different species.

Key words: potato germplasm, *Solanum etuberosum*, molecular cytogenetics.

Résumé : Quatre hybrides somatiques dérivés d'une espèce sauvage diploïde, *Solanum etuberosum*, et le clone 463-4 de *Solanum* (diploïde et tubereux), cinq progénitures BC_1 et trois plantes BC_2 ont été analysés par hybridation génomique *in situ* (GISH). Aucun des quatre hybrides somatiques ne présentait la constitution chromosomique attendue, c'est-à-dire 24 chromosomes de chaque parent. Les hybrides avaient perdu un chromosome soit du *S. etuberosum* soit du parent 463-4. Trois plantes BC_1 possédaient précisément un jeu de chromosomes du *S. etuberosum*. Les deux autres BC_1 comptaient un chromosome du *S. etuberosum* en plus ou en moins, vraisemblablement parce que l'hybride dont ils étaient dérivés en possédait un en plus ou en moins. La présence d'un jeu complet ou presque complet de chromosomes du *S. etuberosum* chez toutes les plantes BC_1 suggère un appariement et une ségrégation préférentiels des chromosomes du *S. etuberosum* chez les hybrides somatiques. Deux des trois plantes BC_2 comptaient 52 chromosomes, une déviation significative par rapport au nombre attendu (48). Ces résultats suggèrent un piètre appariement entre les chromosomes du *S. etuberosum* et du *S. tuberosum* chez les plantes BC_1. La présente étude montre l'importance de combiner les analyses GISH et les analyses de marqueurs moléculaires en vue de la caractérisation détaillée de germoplasme végétal contenant des chromosomes provenant d'espèces différentes.

Mots clés : germoplasme de pomme de terre, *Solanum etuberosum*, cytogénétique moléculaire.

[Traduit par la Rédaction]

Introduction

The non-tuber-bearing wild species *Solanum etuberosum* ($2n = 2x = 24$) is immune or highly resistant to potato virus Y (PVY), potato virus X (PVX), and potato leaf roll virus (PLRV), and is tolerant to frost (Hanneman and Bamberg 1986). However this species cannot be readily crossed with cultivated potato (*S. tuberosum*) because of their differences in endosperm balance number (EBN) (Johnston and Hanneman 1982). To incorporate the valuable traits into cultivated potato, somatic hybrids between *S. etuberosum* and a sexual hybrid between a haploid potato line and *S. berthaultii* were produced by protoplast fusion (Novy and Helgeson 1994*a*). These somatic hybrids bear tubers (Novy and Helgeson 1994*a*) and are resistant to PVY (Novy and

Corresponding Editor: P.B. Moens.

Received January 8, 1999. Accepted April 5, 1999.

F. Dong and J. Jiang.[1] Department of Horticulture, University of Wisconsin—Madison, Madison, WI 53706, U.S.A.
R.G. Novy. Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, U.S.A.
J.P. Helgeson. Agricultural Research Service, U.S. Department of Agriculture and Department of Plant Pathology, University of Wisconsin—Madison, Madison, WI 53706, U.S.A.

[1]Author to whom all correspondence should be addressed (e-mail: jjiang1@facstaff.wisc.edu).

Helgeson 1994*b*). The BC_1 progenies derived from the somatic hybrids showed marked improvements in tuber characteristics and three of the five BC_1 plants were resistant to PVY (Novy and Helgeson 1994*a*, 1994*b*).

An effective technique for identifying *S. etuberosum* chromatin is essential to track the introgression of *S. etuberosum* chromosome(s) or chromosome segment(s) carrying the virus resistance genes into cultivated potato. By restriction fragment length polymorphism (RFLP) analysis, Novy and Helgeson (1994*a*) demonstrated that the somatic hybrids and their backcross progenies contained some portion of both the wild species and potato chromosomes based on the polymorphism of hybridization bands from gel-blot hybridization. However, the results from molecular marker analyses only reveal very fine chromosomal regions. A large number of markers that represent different chromosomal regions would have to be used to analyze a complete chromosome. Chromosomes in *Solanum* species are small and are similar in size. Classical banding techniques do not give a sufficient number of characteristic bands for reliable chromosomal identification (Mok et al. 1974). Therefore, conventional cytogenetic techniques will not be practical for identifying *S. etuberosum* chromosomes in the potato background.

GISH (genomic in situ hybridization) is a technique used to differentiate chromosomes from different species by DNA in situ hybridization. This technique is effective in distinguishing chromosomes from different plant genomes (Jiang and Gill 1994). Wilkinson et al. (1995) demonstrated that GISH can be applied to the small chromosomes from the *Solanum* species. In this report we analyzed the chromosomal constitutions of four somatic hybrids derived from *S. etuberosum*, *S. tuberosum*, and *S. berthaultii* and several corresponding backcross progenies by GISH. We demonstrated that combining molecular marker techniques with GISH analysis provides a comprehensive characterization of plant germplasm containing chromosomes from different species.

Materials and methods

Plant materials

The four somatic hybrids used in the present study were from protoplast fusion of a PVY-resistant *S. etuberosum* clone of PI 245939 and a diploid hybrid 463-4, which was derived from a cross between *S. tuberosum* haploid ($2n = 2x = 24$) clone US-W 730 and an *S. berthaultii* ($2n = 2x = 24$) clone (PI 265857) (Novy and Helgeson 1994*a*). The first backcross progenies were produced by pollinating the somatic hybrids with pollens from tetraploid *S. tuberosum* cultivars Katahdin or Atlantic. One of the BC_1 clone P2-3 was further backcrossed to 'Atlantic' to produce the second backcross progenies.

Genomic in situ hybridization

Genomic DNA was isolated from *S. etuberosum* (PI 245939) and *S. tuberosum* cultivar Katahdin by grinding 5 g of young leaf tissue in liquid nitrogen and then mixing the powder with 15 mL of 2× CTAB (hexadecyltrimethylammonium bromide) solution. After incubation at 60°C for 1 h, an equal volume of chloroform–isoamyl alcohol (24:1) was added, and the mixture was centrifuged for 10 min at 10 000 rpm. The resultant supernatant was filtered through miracloth (Sigma) and precipitated in an equal volume of cold isopropanol. The DNA was then pelleted, washed with 70% ethanol, dried, and resuspended in TE buffer. In GISH analysis, the *S. etuberosum* genomic DNA was labeled with biotin-16-dUTP (Boehringer Mannheim) by standard nick-translation reactions. Potato genomic DNA, sheared to approximately 500 base pairs (bp), was added to the hybridization mixture to block cross-hybridization of the *S. etuberosum* probe to potato chromosomes. Plasmid pTa71 was used to detect the nucleolar organizer regions (NORs). pTa71 was cloned from wheat and contains the coding sequences for 18S-5.8S-26S rRNA genes (Gerlach and Bedbrook 1979).

Root tips were harvested from young greenhouse-grown plants of the somatic hybrids and backcross progenies, pretreated in 0.05% colchicine at 4°C for 5 h, fixed in a 3:1 solution of ethanol – acetic acid for 1 week, and squashed on glass slides with 45% acetic acid. The GISH technique was according to published protocols (Le et al. 1989; Schwarzacher et al. 1989) with minor modifications for potato chromosomes. The slide-bound chromosomal DNA was dehydrated in ethanol and denatured in 70% formamide at 80°C for 1.5 min. Ten microlitres of denatured hybridization mixture containing 20 ng of labeled probe DNA, approximately 1 µg of blocking DNA, 50% formamide, 10% dextran sulfate, 2× SSC, and 10 µg of sheared salmon sperm DNA was applied to each slide. After 24 h hybridization at 37°C and 15 min posthybridization washing in 2× SSC at 42°C, the hybridization signals were detected with a fluorescein isothiocyanate (FITC)-conjugated anti-biotin antibody (Vector). Propidium iodide (PI) in an antifade solution (Vector) was used to counterstain the chromosomes.

After hybridization and detection, slides were examined under an Olympus BX60 fluorescence microscope. Counterstained chromosomes and hybridization signals were captured separately using a SenSys CCD (charge-coupled device) camera (Photometrics), and then merged using an IPLab Spectrum software (Signal Analytics, v. 3.1.1).

Results and discussion

Somatic hybrids

The somatic hybrids were previously characterized using 25 RFLP probes (with 2–3 probes on each of the 12 chromosomes) which were polymorphic between the two fusion parents *S. etuberosum* and clone 463-4 (Novy and Helgeson 1994*a*). Based on the presence of the diagnostic bands for the two parents, it was inferred that both parental genomes were present in all the 14 somatic hybrids analyzed, with 5 somatic hybrids missing 463-4-specific band(s) from one or two probes (Novy and Helgeson 1994*a*). Since the RFLP data can only reveal the defined regions containing the markers analyzed, markers were chosen which were well-separated on the potato linkage groups (Novy and Helgeson 1994*a*). However, the presence of a marker does not distinguish between one or two copies of a particular chromosome. Also, the absence of a marker could be the result of a deletion rather than missing a complete chromosome. For that reason, it was of interest to analyze these materials using the GISH technique.

Four somatic hybrids (Table 1) were analyzed by GISH. *Solanum etuberosum* genomic DNA was labeled as a probe to hybridize in situ to the metaphase chromosomes from the somatic hybrids. By adjusting the hybridization conditions, especially the ratio of probe DNA and blocking DNA, chromosomes from the two fusion parents *S. etuberosum* and clone 463-4 were well differentiated in colors, which gave a direct and unambiguous detection of *S. etuberosum* chromosomes (Figs. 1A and 1B).

Table 1. The chromosome constitutions of somatic hybrids and their backcrossed progenies.

Materials	Sources	No. of lost markers[a]	Chromosome constitutions		
			Total no.	From S.TB.[b]	From S.E.[c]
Somatic hybrids					
2-3-10A	16-1[d] + 463-4[e]	0	48	23	25
2-7-4A	16-1 + 463-4	1	48	24	24
2-7-4D	16-1 + 463-4	0	47	23	24
2-9-3B	16-1 + 463-4	0	48	25	23
BC_1 plants					
P2-1	2-9-3B × 'Katahdin'	0	48	36	12
P2-2	2-3-10A × 'Katahdin'	0	49	36	13
P2-3	2-9-3B × 'Atlantic'	3	48	37	11
P2-4	2-7-4D × 'Katahdin'	—[f]	48	36	12
P2-5	2-7-4A × 'Katahdin'	—	48	36	12
BC_2 plants					
5-31-5	P2-3 × 'Atlantic'	—	52	46	6
6-5-5	P2-3 × 'Atlantic'	—	48	41	7
6-21-3	P2-3 × 'Atlantic'	—	52	44	8

[a]Among the 25 RFLP probes analyzed, the diagnostic hybridization band(s) for either fusion parent was not detected from a given RFLP probe or probes.
[b]*Solanum tuberosum* and (or) *S. berthaultii*.
[c]*Solanum etuberosum.*
[d]The *S. etuberosum* line (PI 245939) used in somatic fusion.
[e]The hybrid clone between diploid *S. tuberosum* line US-W 730 and *S. berthaultii* used in somatic fusion.
[f]Data not available.

Based on RFLP analysis, somatic hybrids 2-3-10A, 2-9-3B, and 2-7-4D contained at least one complete set of chromosomes from each fusion parent (Novy and Helgeson 1994*a*). However, the GISH results revealed that 2-3-10A had 25 chromosomes from *S. etuberosum* and 23 chromosomes from clone 463-4 (Fig. 1A); 2-9-3B had 23 from *S. etuberosum* and 25 from clone 463-4 (Fig. 1B); and 2-7-4D had 24 from *S. etuberosum* and 23 from clone 463-4 (data not shown). None of these hybrids had the exact chromosome constitution expected, i.e., 24 chromosomes from each fusion parent (Table 1). Either a chromosome from *S. etuberosum* or one from clone 463-4 was lost or gained during the generation of the somatic hybrids. The GISH results indicated that missing one of the two members of a particular homologue was difficult to detect by RFLP analysis.

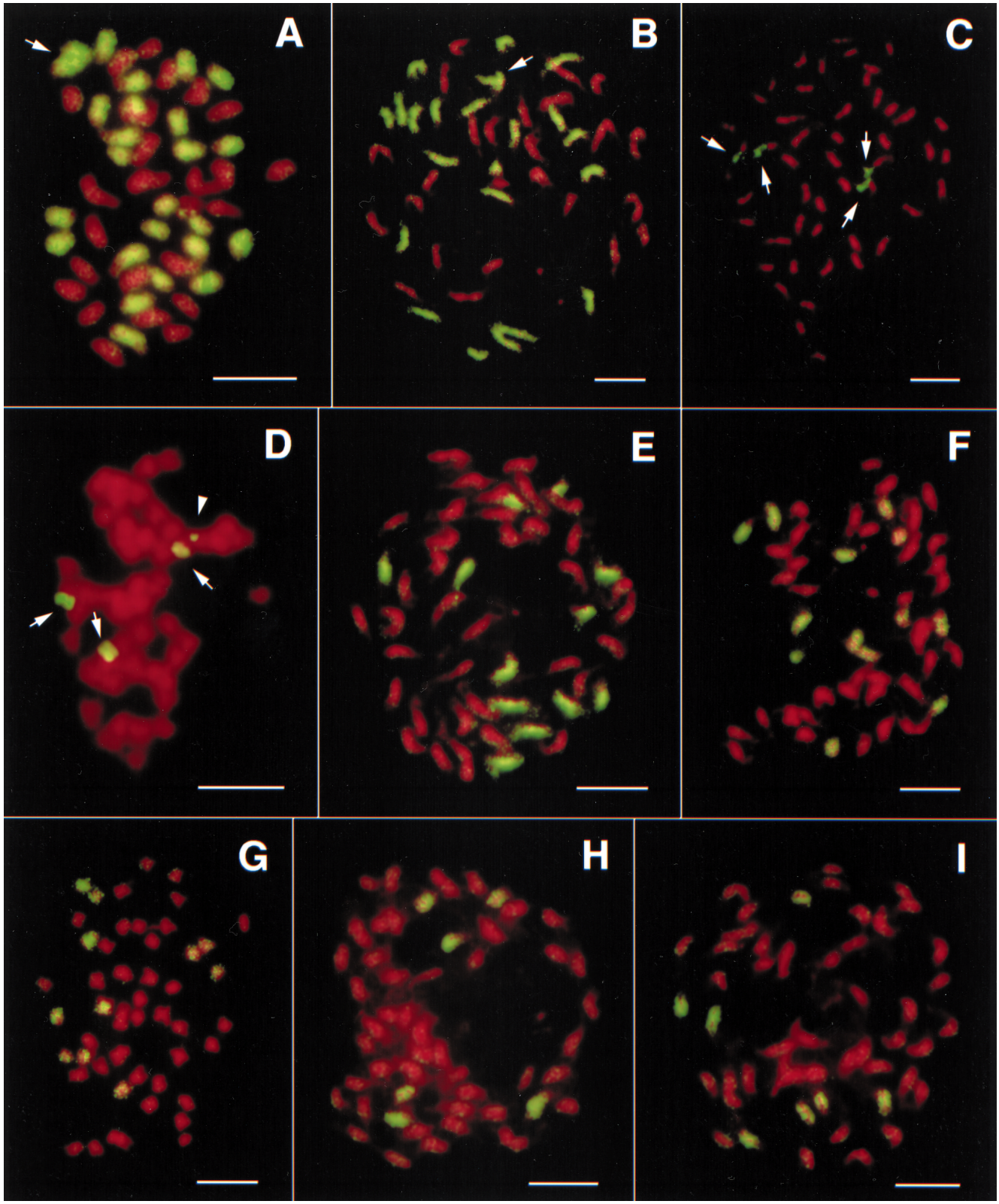
RFLP analysis indicated that somatic hybrid 2-7-4A lost a 463-4-specific band from probe TG68 (Novy and Helgeson 1994*a*). GISH analysis showed that 24 of the 48 chromosomes from 2-7-4A were labeled by the *S. etuberosum* probe (Table 1), suggesting that this somatic hybrid did not lose any chromosome from the 463-4 parent. However, it is possible that a small chromosome segment containing marker TG68 was deleted in the hybrid, and this segment is too small to detect by GISH.

The differences between results with RFLP and GISH analyses with reference to genetic constitutions of somatic hybrids demonstrates the importance of the combined application of these two methods. While RFLP analysis focus on fine chromosomal regions, GISH provides information at the chromosome level, but may not detect small deletions. The combination of the two techniques resulted in a more thorough characterization of these materials.

In the somatic hybrids between *S. tuberosum* and *S. phureja*, Pijnacker et al. (1987) noticed preferential elimination of the two nucleolus organizing (NOR) chromosomes. We were interested to find out whether the missing chromosomes, either from *S. etuberosum* or from 463-4, in the somatic hybrids are NOR chromosomes. The four somatic hybrids were analyzed by in situ hybridization using DNA probe pTa71, which contains the coding sequences for 5.8S–18S–26S rRNA genes. Somatic hybrids 2-3-10A and 2-7-4D had four strong hybridization signals, indicating the presence of four NOR chromosomes as expected (Fig. 1C for 2-3-10A, data not shown for 2-7-4D). This result suggests no preferential elimination of NOR chromosomes. Somatic hybrids 2-9-3B (Fig. 1D) and 2-7-4A (data not shown) showed three large and one relatively small hybridization signals. It was not known if the smaller signal on one of the four NOR chromosomes resulted from the chromosome rearrangement(s) as reported in *S. tuberosum* + *S. phureja* somatic hybrids (Pijnacker et al. 1987) or from delayed condensation as observed in *S. tuberosum* + *S. brevidens* somatic hybrids (McGrath and Helgeson 1998).

The first backcross progenies

The chromosomes from the two fusion parents belong to A (*S. tuberosum* and *S. berthaultii*) and E (*S. etuberosum*) genomes, respectively (Matsubayashi 1991). If we assume that the chromosomes from the two parents pair preferentially and segregate independently, we would expect most of the BC_1 plants to have 12 chromosomes from *S. etuberosum* and 36 chromosomes from potato. Five BC_1 plants were analyzed by GISH (Table 1). Three BC_1 plants P2-1, P2-4, and P2-5, had the expected chromosome constitutions (Fig. 1E for P2-1, data not shown for the others). BC_1 plant P2-2, had



49 chromosomes, with 13 chromosomes from *S. etuberosum* (Fig. 1F). The somatic hybrid parent of this plant, 2-3-10A, has 25 *S. etuberosum* chromosomes (Fig. 1A; Table 1). It is likely that the extra *S. etuberosum* chromosome in 2-3-10A was transmitted to P2-2. BC_1 plant P2-3 had 48 chromosomes, 11 of which were from *S. etuberosum* (Fig. 1G). The somatic hybrid parent of this plant, 2-9-3B, has only 23 *S. etuberosum* chromosomes (Fig. 1B; Table 1). Thus, the

Fig. 1. Somatic metaphase chromosome spreads from somatic hybrids and their backcross progenies after in situ hybridization using *S. etuberosum* genomic DNA (A, B, E–I) or pTa71 (C and D) as probes. Hybridization signals were detected by FITC-conjugated antibodies (green), and chromosomes were counterstained with PI (red). (A) Somatic hybrid 2-3-10A, 2*n* = 48, with 25 chromosomes from *S. etuberosum*. The arrow points to two overlapping *S. etuberosum* chromosomes. (B) Somatic hybrid 2-9-3B, 2*n* = 48, with 23 chromosomes from *S. etuberosum*. The arrow points to two overlapping *S. etuberosum* chromosomes. (C) 2-3-10A, with four strong NOR signals (arrows). (D) 2-9-3B, with three strong (arrows) plus one weak (arrowhead) NOR signals. (E) BC_1 clone P2-1, 2*n* = 48, with 12 *S. etuberosum* chromosomes. (F) BC_1 clone P2-2, 2*n* = 49, with 13 *S. etuberosum* chromosomes. (G) BC_1 clone P2-3, 2*n* = 48, with 11 *S. etuberosum* chromosomes. (H) BC_2 clone 5-31-5, 2*n* = 52, with six chromosomes from *S. etuberosum*. (I) BC_2 clone 6-5-5, 2*n* = 48, with seven *S. etuberosum* chromosomes. All bars are 10 μm.

missing chromosome in P2-3 is likely the same one lost in 2-9-3B.

Three of the BC_1 plants P2-1, P2-2, and P2-3 were analyzed previously with RFLPs (Novy and Helgeson 1994*a*). Loss of *S. etuberosum* specific band(s) was not observed for clones P2-1 and P2-2 from analysis of 25 RFLP probes. Chromosome constitutions of these two clones as indicated by GISH analysis supports the RFLP results. Clone P2-3 missed three RFLP markers specific to chromosomes 2, 7, and 11. However, the other markers specific to those three chromosomes were present in this plant, suggesting that at least part of these three chromosomes are present in this clone. GISH results showed that there were only 11 *S. etuberosum* chromosomes in P2-3. The contradictory results from the two different techniques suggest that one or few recombinant chromosomes may be present in this clone. The *S. etuberosum* chromosomal segments of these chromosomes are possibly too small to be detected using the GISH technique.

Among the five BC_1 plants investigated, three have exactly one set of *S. etuberosum* chromosomes. The other two plants either had one extra or one fewer *S. etuberosum* chromosome, but this can be well explained by the chromosomal constitutions of their somatic hybrid parents. The presence of a single set, or close to a single set, of *S. etuberosum* chromosomes in all the BC_1 plants indicates a preferential pairing and independent segregation of the *S. etuberosum* chromosomes in the somatic hybrids.

The second backcross progenies

Three BC_2 plants, which were produced from BC_1 clone P2-3 by using a tetraploid *S. tuberosum* cultivar Atlantic as a recurrent parent, were analyzed by GISH (Table 1). The 11 *S. etuberosum* chromosomes from P2-3 segregated among the BC_2 progenies. Six, seven, and eight *S. etuberosum* chromosomes were detected in BC_2 clones 5-31-5 (Fig. 1H), 6-5-5 (Fig. 1I), and 6-21-3 (data not shown), respectively. All three of the BC_2 progenies examined with GISH were successfully used as parents this past winter to produce the BC_3 generation. Currently in the greenhouse, we have 233 BC_3 progenies representing eight different families. Our ultimate goal is to develop potato clones containing a single *S. etuberosum* chromosome. Once a complete set of 12 such monosomic addition or substitution lines is established, it will be possible to locate the virus resistance genes on specific *S. etuberosum* chromosome(s). Such clones can also be used as germplasm directly in potato-breeding programs.

Solanum brevidens is also a non-tuber-bearing diploid species and shares the same genome as *S. etuberosum* (Matsubayashi 1991). Pairing between potato and *S. brevidens* chromosomes was observed based on cytological observation (Williams et al. 1993) and was also indicated based on both RFLP and RAPD (randomly amplified polymorphic DNA) analyses (Williams et al. 1993; McGrath et al. 1994, 1996). Thus it is expected that the *S. etuberosum* chromosomes will have a similar probability to pair with *S. tuberosum* chromosomes, especially in BC_1 plants in which the single set of *S. etuberosum* chromosomes have no chance for preferential pairing. However, if the 11 *S. etuberosum* chromosomes paired well with their partners from *S. tuberosum* or *S. berthaultii* in the BC_1 plant P2-3, it would be expected the chromosome numbers of the BC_2 clones to be close to 48. However, two of the three BC_2 clones analyzed have 52 chromosomes (Table 1; Figs. 1H and 1I). The chromosome number deviations from the expectation suggest that the pairing of *S. etuberosum* chromosomes with their partners from potato is limited. The chromosomes from potato in BC_1 plants may preferentially pair with each other, resulting in *S. etuberosum* chromosomes as univalents. Random segregation of the univalents may contribute to the chromosome number deviation of BC_2 plants. Another interesting observation was that no recombinant and (or) translocation chromosomes were detected in the three BC_2 plants. This result again suggests a poor paring between *S. etuberosum* and potato chromosomes in the BC_1 plants, although it is possible that recombinant chromosomes with a very small chromosome fragment shift were actually present in the BC_2 plants, but were undetected by the GISH technique.

Acknowledgements

This research is supported by Hatch Funds 142-3935, 142-D395, and 142-N395 to J.J.

References

Gerlach, W.L., and Bedbrook, J.R. 1979. Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res. **7**: 1869–1885.

Hanneman, R.E., Jr., and Bamberg, J.B. 1986. Inventory of tuber-bearing *Solanum* species. Bulletin 533, Research Division, College of Agriculture and Life Sciences, The University of Wisconsin—Madison, Madison, Wis. 216 pp.

Jiang, J., and Gill, B.S. 1994. Non-isotopic in situ hybridization and plant genome mapping: The first 10 years. Genome, **37**: 717–725.

Johnston, S.A., and Hanneman, R.E., Jr. 1982. Manipulations of endosperm balance number overcomes crossing barriers between diploid *Solanum* species. Science (Washington, D.C.), **217**: 446–448.

Le, H.T., Armstrong, K.C., and Miki, B. 1989. Detection of rye DNA in wheat–rye hybrids and wheat translocation stocks using total genomic DNA as a probe. Plant Mol. Biol. Rep. **7**: 150–158.

Matsubayashi, M. 1991. Phylogenetic relationships in the potato and its related species. *In* Chromosome engineering in plants. Genetics, breeding, evolution, Part B. *Edited by* T. Tsuchiya and P.K. Gupta. Elsevier Science Publishers B.V., Amsterdam. pp. 93–118.

McGrath, J.M., and Helgeson, J.P. 1998. Differential behavior of *Solanum brevidens* ribosomal DNA loci in a somatic hybrid and its progeny with potato. Genome, **41**: 435–439.

McGrath, J.M., Wielgus, S.M., Uchytil, T.F., Kim-Lee, H., Haberlach, G.T., Williams, C.E., and Helgeson, J.P. 1994. Recombination of *Solanum brevidens* chromosomes in the second backcross generation from a somatic hybrid with *S. tuberosum*. Theor. Appl. Genet. **88**: 917–924.

McGrath, J.M., Wielgus, S.M., and Helgeson, J.P. 1996. Segregation and recombination of *Solanum brevidens* synteny groups in progeny of somatic hybrids with *S. tuberosum*: Intragenomic equals or exceeds intergenomic recombination. Genetics, **142**: 1335–1348.

Mok, D.W.S., Lee, H.K, and Peloquin, S.J. 1974. Identification of potato chromosomes with Giemsa. Am. Potato J. **51**: 337–341.

Novy, R.G., and Helgeson, J.P. 1994*a*. Somatic hybrids between *Solanum etuberosum* and diploid, tuber bearing *Solanum* clones. Theor. Appl. Genet. **89**: 775–782.

Novy, R.G., and Helgeson, J.P. 1994*b*. Resistance to potato virus Y in somatic hybrids between *Solanum etuberosum* and *S. tuberosum* × *S. berthaultii* hybrid. Theor. Appl. Genet. **89**: 783–786.

Pijnacker, L.P., Ferwerda, M.A., Puite, K.J., and Roest, S. 1987. Elimination of *Solanum phureja* nucleolar chromosomes in *S. tuberosum* + *S. phureja* hybrids. Theor. Appl. Genet. **73**: 878–882.

Schwarzacher, T., Leitch, A.R., Bennett, M.D., and Heslop-Harrison, J.S. 1989. In-situ localization of parental genomes in a wild hybrid. Ann. Bot. **64**: 315–324.

Wilkinson, M.J., Bennett, S.T., Clulow, S.A., Allainguillaume, J., Harding, K., and Bennett, M.D. 1995. Evidence for somatic translocation during potato dihaploid induction. Heredity, **74**: 146–151.

Williams, C.E., Wielgus, S.M., Haberlach, G.H., Guenther, C., Kim-Lee, H., and Helgeson, J.P. 1993. RFLP analysis of chromosomal segregation in progeny from an interspecific hexaploid somatic hybrid between *Solanum brevidens* and *Solanum tuberosum*. Genetics, **135**: 1167–1173.